

NSWER 20 OF 22 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.

on STN

ACCESSION NUMBER: 87058717 EMBASE  
DOCUMENT NUMBER: 1987058717  
TITLE: In vitro packaging into phage T4 particles and specific  
recircularization of phage lambda DNAs.  
AUTHOR: Black L.W.  
CORPORATE SOURCE: Department of Biochemistry, University of Maryland Medical  
School, Baltimore, MD 21201, United States  
SOURCE: Gene, (1986) 46/1 (97-101).  
CODEN: GENED6  
COUNTRY: Netherlands  
DOCUMENT TYPE: Journal  
FILE SEGMENT: 004 Microbiology  
022 Human Genetics  
047 Virology  
LANGUAGE: English

AB Concatemeric phage  $\lambda$ imm434 DNA packaged in vitro into phage T4 particles produced plaques on a selective host. Moreover,  $\lambda$  DNA containing a pBR322 derivative flanked by the  $\lambda$  attL and attR sites could be specifically recircularized by excisive  $\lambda$  recombination to yield the pBR322 derivative. A host deficient in generalized recombination and containing a defective  $\lambda$ cIts prophage which provided Int and Xis proteins was the recipient for this plasmid derivative carried by T4. Such a T4- $\lambda$  hybrid may potentially allow almost one T4 headful of donor DNA (166 kb) to be packaged and recircularized.

NSWER 1 OF 22 MEDLINE on STN  
ACCESSION NUMBER: 1999377766 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 10448644  
TITLE: Efficient generation of recombinant adenoviral vectors by  
**Cre-lox recombination** in vitro.  
AUTHOR: Aoki K; Barker C; Danthinne X; Imperiale M J; Nabel G J  
CORPORATE SOURCE: Howard Hughes Medical Institute, University of Michigan  
Medical Center, Department of Internal Medicine and  
Biological Chemistry, Ann Arbor 48109-0650, USA.  
CONTRACT NUMBER: GM34902 (NIGMS)  
SOURCE: Molecular medicine (Cambridge, Mass.), (1999 Apr) 5 (4)  
224-31.

PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199909  
ENTRY DATE: Entered STN: 19990925  
Last Updated on STN: 19990925  
Entered Medline: 19990913

AB BACKGROUND: Although recombinant adenovirus vectors are attractive for use in gene expression studies and therapeutic applications, the construction of these vectors remains relatively time-consuming. We report here a strategy that simplifies the production of adenoviruses using the Cre-**loxP** system. MATERIALS AND METHODS: Full-length recombinant adenovirus DNA was generated in vitro by Cre-mediated recombination between **loxP** sites in a linearized shuttle plasmid containing a transgene and adenovirus genomic DNA. RESULTS: After transfection of Cre-treated DNA into 293 cells, replication-defective viral vectors were rapidly obtained without detectable wild-type virus. CONCLUSION: This system facilitates the development of recombinant adenoviral vectors for basic and clinical research.

L16 ANSWER 2 OF 22 MEDLINE on STN  
ACCESSION NUMBER: 97184512 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 9032314  
TITLE: Construction of adenovirus vectors through Cre-**lox** recombination.  
AUTHOR: Hardy S; Kitamura M; Harris-Stansil T; Dai Y; Phipps M L  
CORPORATE SOURCE: Somatix Therapy Corporation, Alameda, California  
94501-1034, USA.. shardy@apple.somatix.com  
SOURCE: Journal of virology, (1997 Mar) 71 (3) 1842-9.  
Journal code: 0113724. ISSN: 0022-538X.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-U62024  
ENTRY MONTH: 199703  
ENTRY DATE: Entered STN: 19970327  
Last Updated on STN: 19970327  
Entered Medline: 19970318

AB Two barriers prevent adenovirus-based vectors from having wide application. One is the difficulty of making new adenoviruses, and the second is the strong immunological reaction to viral proteins. Here we describe uses of Cre-**lox** recombination to overcome these problems. First, we demonstrate a simple method for constructing E1-substituted adenoviruses. Second, we demonstrate a method to construct adenovirus vectors carrying recombinant genes in place of all of the viral genes, so-called gutless adenovirus vectors. The pivotal feature in each method is the use of a negatively selected adenovirus named ps15. We

engineered a *cis*-acting selection into psi5 by flanking its packaging site with **loxP** sites. When psi5 was grown in cells making a high level of Cre **recombinase**, the packaging site was deleted by **recombination** and the yield of psi5 was reduced to 5% of the wild-type level. To make a new El-substituted virus, we used psi5 as a donor virus and recombined it with a **shuttle vector** via a **loxP** site. The resulting recombinant virus has a single **loxP** site next to the packaging site and therefore outgrows psi5 in the presence of Cre **recombinase**. To make a gutless virus, we used psi5 as a helper virus. The only viral sequences included in the gutless vector are those needed in *cis* for its replication and packaging. We found that a **loxP** site next to the packaging site of the gutless virus was necessary to neutralize homologous **recombination** between psi5 and the gutless viruses within their packaging domains.

L16 ANSWER 3 OF 22 MEDLINE on STN  
ACCESSION NUMBER: 97105907 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 8948655  
TITLE: Rapid construction in yeast of complex targeting vectors for gene manipulation in the mouse.  
AUTHOR: Storck T; Kruth U; Kolhekar R; Sprengel R; Seeburg P H  
CORPORATE SOURCE: Center for Molecular Biology (ZMBH), University of Heidelberg, Germany.. storck@sun0.urz.uni-heidelberg.de  
SOURCE: Nucleic acids research, (1996 Nov 15) 24 (22) 4594-6.  
Journal code: 0411011. ISSN: 0305-1048.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-U63018; GENBANK-U63120  
ENTRY MONTH: 199701  
ENTRY DATE: Entered STN: 19970219  
Last Updated on STN: 19970219  
Entered Medline: 19970117

AB Targeting vectors for embryonic stem (ES) cells typically contain a mouse gene segment of >7 kb with the neo gene inserted for positive selection of the targeting event. More complex targeting vectors carry additional genetic elements (e.g. lacZ, **loxP**, point mutations). Here we use homologous **recombination** in yeast to construct targeting vectors for the incorporation of genetic elements (GEs) into mouse genes. The precise insertion of GEs into any position of a mouse gene segment cloned in an *Escherichia coli*/yeast **shuttle vector** is directed by short recombinogenic arms (RAs) flanking the GEs. In this way, complex targeting vectors can be engineered with considerable ease and speed, obviating extensive gene mapping in search for suitable restriction sites.

L16 ANSWER 4 OF 22 MEDLINE on STN  
ACCESSION NUMBER: 96106831 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 7503713  
TITLE: Efficient regulation of gene expression by adenovirus vector-mediated delivery of the CRE **recombinase**.  
AUTHOR: Sakai K; Mitani K; Miyazaki J  
CORPORATE SOURCE: Department of Disease-related Gene Regulation Research (Sandoz), University of Tokyo, Japan.  
SOURCE: Biochemical and biophysical research communications, (1995 Dec 14) 217 (2) 393-401.  
Journal code: 0372516. ISSN: 0006-291X.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199601

ENTRY DATE: Entered STN: 19960217  
Last Updated on STN: 19960217  
Entered Medline: 19960118

AB We have constructed an E1-defective adenovirus (Ad) **vector** designated AdCAG-Cre containing the Cre **recombinase** gene derived from bacteriophage P1 under control of the cytomegalovirus immediate early enhancer-chicken beta-actin **hybrid** (CAG) promoter. We examined the Cre-**loxP**-based **recombination** by this Ad vector in C2C12 cells bearing a reporter gene construct CAG-CAT-Z, which directs expression of the *E. coli* lacZ gene upon Cre-mediated excision of the CAT gene located between the CAG promoter and the lacZ gene. Nearly 100% of these cells were shown to start to produce beta-galactosidase after infection with the AdCAG-Cre vector at MOI 100. On the basis of this result, we discuss the possible use of the AdCAG-Cre vector to manipulate the gene expression in mammalian cells.

L16 ANSWER 5 OF 22 MEDLINE on STN  
ACCESSION NUMBER: 95291341 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 7773312  
TITLE: A novel Ti-plasmid-convertible lambda phage vector system suitable for gene isolation by genetic complementation of *Arabidopsis thaliana* mutants.  
AUTHOR: Fuse T; Kodama H; Hayashida N; Shinozaki K; Nishimura M; Iba K  
CORPORATE SOURCE: Department of Biology, Faculty of Science, Kyushu University, Fukuoka, Japan.  
SOURCE: Plant journal : for cell and molecular biology, (1995 May) 7 (5) 849-56.  
JOURNAL code: 9207397. ISSN: 0960-7412.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199507  
ENTRY DATE: Entered STN: 19950720  
Last Updated on STN: 19950720  
Entered Medline: 19950713

AB A new lambda phage vector system, lambda TI, has been constructed to facilitate genetic complementation of higher plant mutations. The lambda TI vectors are stable, and by using the Cre-**lox** site-specific **recombination**, are automatically convertible into Ti-**plasmid binary** vectors which are capable of expressing genes in higher plants. Two lambda TI vectors were constructed: (i) lambda TI1, which can generate a Ti-plasmid that contains the cauliflower mosaic virus (CaMV) 35S promoter and is suitable for the expression of cDNA in transformed plants and (ii) lambda TI2, which can generate a Ti-plasmid with the multicloning site (MCS). cDNA and genomic libraries, which were constructed from the cruciferous plant *Arabidopsis thaliana* in these lambda TI vectors, can be probed by large DNA fragments of more than 100 kb, such as yeast artificial chromosomes (YACs), enabling the direct screening of the clones in the chromosome region containing a specified genetic locus. These libraries will certainly become powerful tools for the genetic complementation of *Arabidopsis* mutant phenotypes by quickly providing transformation-competent clones.

L16 ANSWER 6 OF 22 MEDLINE on STN  
ACCESSION NUMBER: 94355044 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 8074871  
TITLE: Lambda/plasmid vector construction by in vivo cre/  
**lox**-mediated **recombination**.  
AUTHOR: Brunelli J P; Pall M L  
CORPORATE SOURCE: Washington State University, Pullman 99164-4234.  
SOURCE: BioTechniques, (1994 Jun) 16 (6) 1060-4.

Journal code: 8306785. ISSN: 0736-6205.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Report; (TECHNICAL REPORT)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199410  
ENTRY DATE: Entered STN: 19941013  
Last Updated on STN: 19941013  
Entered Medline: 19941006

AB Lambda/**plasmid** **hybrid** vectors have been previously constructed in which the **plasmid** sequences are separated from flanking lambda arms by **lox** sites. The **lox** sequence is the substrate of Cre-mediated site-specific **recombination**, allowing easy excision of plasmid sequences (automatic subcloning). We have developed a simple procedure to construct other such lambda **hybrid** vectors using *in vivo* **cre/lox**-mediated **recombination** to exchange new plasmids for plasmids previously incorporated into lambda/**plasmid** hybrids. Because **hybrid** vectors both with and without lacZ alpha **plasmid** sequences are available, producing either blue or clear plaques, respectively, the new lambda **hybrid** vectors can be distinguished from the parental hybrids by blue/clear plaque screening. This procedure has been successfully used to construct ten hybrid vectors. It generates new lambda/**plasmid** **hybrid** vectors, without ligation or lambda packaging, which retain the property of automatic subcloning.

L16 ANSWER 7 OF 22 MEDLINE on STN  
ACCESSION NUMBER: 92235813 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 1569546  
TITLE: The mcm2 mutation of yeast affects replication, rather than segregation or amplification of the two micron plasmid.  
AUTHOR: Maiti A K; Sinha P  
CORPORATE SOURCE: Department of Biochemistry, Bose Institute, Calcutta, India.  
SOURCE: Journal of molecular biology, (1992 Apr 5) 224 (3) 545-58.  
Journal code: 2985088R. ISSN: 0022-2836.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199205  
ENTRY DATE: Entered STN: 19920612  
Last Updated on STN: 19920612  
Entered Medline: 19920526

AB We have studied the maintenance of the endogenous two micron (2 mu) plasmid in a strain of yeast carrying the nuclear mutation mcm2. This mutation, earlier shown to affect the maintenance of yeast minichromosomes in an ARS-dependent manner, also affected the copy number of the 2 mu plasmid. The effect was more pronounced at 35 degrees C leading to the elimination of the plasmid from the cells cultured at this temperature. The mutant cells could be efficiently cured of the circle by transformation with 2 mu ORI-carrying **hybrid** vectors, an observation consistent with the low copy number of the endogenous **plasmid**. A chromosomal revertant of this mutant for another ARS(ARS1) was found also to confer stability on the 2 mu ORI-carrying minichromosomes and had elevated levels of the endogenous plasmid. The mutation neither affected the segregation nor the amplification process mediated by site-specific **recombination** at **FRT** sites requiring the FLP gene-encoded protein action. ARS131C, an ARS that was unaffected in the mutant at 25 degrees C, could elevate the copy number of a 2 mu **hybrid** **vector** in the mutant cells. In view of these results, some aspects of segregation and copy number control of the endogenous plasmid have been discussed. We propose that the mutation

impairs the 2 mu ORI function, leading to its loss.

L16 ANSWER 8 OF 22 MEDLINE on STN  
ACCESSION NUMBER: 90147639 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 2695072  
TITLE: REP3-derived yeast **shuttle vector**.  
AUTHOR: Schubert U; Gniel D; Lang H  
CORPORATE SOURCE: Central Institute of Microbiology and Experimental Therapy,  
Academy of Sciences of the GDR, Jena.  
SOURCE: Biomedica biochimica acta, (1989) 48 (8) 529-37.  
Journal code: 8304435. ISSN: 0232-766X.  
PUB. COUNTRY: GERMANY, EAST: German Democratic Republic  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199002  
ENTRY DATE: Entered STN: 19900328  
Last Updated on STN: 19900328  
Entered Medline: 19900226

AB A yeast-**shuttle vector** using a XbaI-PstI fragment of the 2 microns DNA 1293 bp in length has been constructed. This sequence spans the REP 3 locus and the origin of replication. Besides the 2 microns DNA derived sequences the resulting yeast **shuttle vector** contains the yeast LEU 2 gene and the Tet' gene of pBR 322. The results demonstrate that the XbaI-PstI fragment is sufficient for proper amplification and partitioning of 2 microns DNA derived yeast-shuttle vectors in yeast. The interruption of the **FRT** site in this type of **vector** seems to prevent any **recombination** between endogenous 2 microns DNA and **hybrid plasmid** molecules. Moreover, the disturbance of the FLP-system has no influence on the copy number of the plasmid stability.

L16 ANSWER 9 OF 22 MEDLINE on STN  
ACCESSION NUMBER: 88314876 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 3045079  
TITLE: Characterization of the rec-1 gene of Haemophilus influenzae and behavior of the gene in Escherichia coli.  
AUTHOR: Setlow J K; Spikes D; Griffin K  
CORPORATE SOURCE: Biology Department, Brookhaven National Laboratory, Upton, New York 11973.  
SOURCE: Journal of bacteriology, (1988 Sep) 170 (9) 3876-81.  
Journal code: 2985120R. ISSN: 0021-9193.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198810  
ENTRY DATE: Entered STN: 19900308  
Last Updated on STN: 19900308  
Entered Medline: 19881006

AB The rec-1 gene of Haemophilus influenzae was cloned into a **shuttle vector** that replicates in Escherichia coli as well as in H. influenzae. The plasmid, called pRec1, complemented the defects of a rec-1 mutant in repair of UV damage, transformation, and ability of prophage to be induced by UV radiation. Although UV resistance and **recombination** were caused by pRec1 in E. coli recA mutants, UV induction of lambda and UV mutagenesis were not. We suggest that the ability of the H. influenzae Rec-1 protein to cause cleavage of repressors but not the **recombinase** function differs from that of the E. coli RecA protein.

L16 ANSWER 10 OF 22 MEDLINE on STN  
ACCESSION NUMBER: 87089725 MEDLINE

DOCUMENT NUMBER: PubMed ID: 3025614  
TITLE: Mating type-like conversion promoted by the 2 micrograms circle site-specific **recombinase**: implications for the double-strand-gap repair model.  
AUTHOR: Jayaram M  
SOURCE: Molecular and cellular biology, (1986 Nov) 6 (11) 3831-7.  
Journal code: 8109087. ISSN: 0270-7306.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198702  
ENTRY DATE: Entered STN: 19900302  
Last Updated on STN: 19990129  
Entered Medline: 19870217

AB Double-strand breaks in DNA are known to promote **recombination** in *Saccharomyces cerevisiae*. Yeast mating type switching, which is a highly efficient gene conversion event, is apparently initiated by a site-specific double-strand break. The 2 micrograms circle site-specific **recombinase**, FLP, has been shown to make double-strand breaks in its substrate DNA. By using a **hybrid** 2 micrograms circle::Tn5 **plasmid**, a portion of which resembles, in its DNA organization, the active (MAT) and the silent (HML) yeast mating type loci, it is shown that FLP mediates a conversion event analogous to mating type switching. Whereas the FLP site-specific **recombination** is not dependent on the RAD52 gene product, the FLP-induced conversion is abolished in a rad52 background. The FLP-promoted conversion *in vivo* can be faithfully reproduced by making a double-stranded gap *in vitro* in the vicinity of the FLP site and allowing the gap to be repaired *in vivo*.

L16 ANSWER 11 OF 22 MEDLINE on STN  
ACCESSION NUMBER: 81237782 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 6265316  
TITLE: Functional analysis of hybrid plasmids carrying genes for lambda site-specific **recombination**.  
AUTHOR: Strizhov N; Soukovatitsin V; Ksenzenko V; Tikhomirova L;  
Bayev A  
SOURCE: Gene, (1980 Dec) 12 (3-4) 201-14.  
Journal code: 7706761. ISSN: 0378-1119.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198109  
ENTRY DATE: Entered STN: 19900316  
Last Updated on STN: 19900316  
Entered Medline: 19810915

AB A number of hybrid plasmids, carrying lambda genes involved in site-specific integrative **recombination**, have been constructed *in vitro*. Analysis of protein synthesis in *Escherichia coli* minicells has shown that Int protein is synthesized only when int gene is expressed constitutively. The plasmids RSF2124::lambda-CD, RSF2124::lambda-Cint-c57, and pInt lambda were able to integrate into the chromosome of *E.coli* at the **attB**. The integration of hybrid plasmids *in vitro* into the genome of bacteria has also been shown for *polA1* strains restricting the autonomous replication of *ColE1* type plasmids. Genetic markers of hybrid plasmids are maintained in *polA1* bacteria for at least 50 generations under nonselective conditions. The Southern blotting experiments using [<sup>32</sup>P]pBR322 DNA and EcoRI fragments of *E. coli* *polA1* chromosome carrying integrated **plasmid** pInt lambda demonstrated that in this strain **hybrid** plasmids can be observed only when integrated into the **attB** of the chromosome according to Campbell's model of integration. In the cells, where autonomous replication of plasmids is

possible, they can be observed both in extrachromosomal and integrated states. The integration of the ColE1 replication origin into the chromosome of bacteria is not lethal for the cells. Only **attP** and the **int** gene of lambda are necessary for the integration of hybrid plasmids under conditions of effective **int** gene expression. If the level of **Int** protein synthesis is high enough, the prophage excision can be observed in the absence of **Xis** product. The six-fold decrease of **Int** protein concentration in the cell (in case of pInt lambda 2 as compared to pInt lambda 1) is critical both for integration and excision.

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on STN

ACCESSION NUMBER: 1999246117 EMBASE  
TITLE: Reversible immortalization of human myogenic cells by site-specific excision of a retrovirally transferred oncogene.  
AUTHOR: Berghella L.; De Angelis L.; Coletta M.; Berarducci B.; Sonnino C.; Salvatori G.; Anthonissen C.; Cooper R.; Butler-Browne G.S.; Mouly V.; Ferrari G.; Mavilio F.; Cossu G.  
CORPORATE SOURCE: Dr. G. Cossu, Dept. of Histol. and Med. Embryol., University of Roma La Sapienza, Via A. Scarpa 14, 00161 Rome, Italy. cossu@axrma.uniromal.it  
SOURCE: Human Gene Therapy, (1 Jul 1999) 10/10 (1607-1617).  
Refs: 41  
ISSN: 1043-0342 CODEN: HGTHE3  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 022 Human Genetics  
026 Immunology, Serology and Transplantation  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB Myogenic cells have a limited life span in culture, which prevents expansion at clinically relevant levels, and seriously limits any potential use in cell replacement or ex vivo gene therapy. We developed a strategy for reversibly immortalizing human primary myogenic cells, based on retrovirus- mediated integration of a wild-type SV40 large-T antigen (Tag), excisable by means of the **Cre-Lox recombination** system. Myogenic cells were transduced with a **vector** (LTTN-**LoxP**) expressing the SV40 Tag under the control of an LTR modified by the insertion of a **LoxP** site in the U3 region. Clonal isolates of Tag-positive cells showed modified growth characteristics and a significantly extended life span, while maintaining a full myogenic potential. Transient expression of **Cre recombinase**, delivered by transfection or adenoviral **vector** transduction, allowed excision of the entire provirus with up to >90% efficiency. Cultures of Cre-treated (Tag-) or untreated (Tag+) myogenic cells were genetically labeled with a lacZ retroviral **vector**, and injected into the regenerating muscle of SCID/bg immunodeficient mice. Tag- cells underwent terminal differentiation in vivo, giving rise to clusters of  $\beta$ - Gal+ **hybrid** fibers with an efficiency comparable to that of control untransduced cells. Tag+ cells could not be detected after injection. Neither Tag+ nor Tag- cells formed tumor in this xenotransplantation model. Reversible immortalization by Tag therefore allows the expansion of primary myogenic cells in culture without compromising their ability to differentiate in vivo, and could represent a safe method by which to increase the availability of these cells for clinical application.

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ACCESSION NUMBER: 96150435 EMBASE  
DOCUMENT NUMBER: 1996150435  
TITLE: Accessory proteins impose site selectivity during ColE1

AUTHOR: Guhathakurta A.; Viney I.; Summers D.  
CORPORATE SOURCE: Department of Genetics, Downing Street, Cambridge CB2 3EH,  
United Kingdom  
SOURCE: Molecular Microbiology, (1996) 20/3 (613-620).  
ISSN: 0950-382X CODEN: MOMIEE  
COUNTRY: United Kingdom  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB The cer-Xer dimer resolution system of **plasmid** ColE1 is highly selective, acting only at sites on the same molecule and in direct repeat. **Recombination** requires the XerCD **recombinase** and accessory proteins ArgR and pepA. The *Escherichia coli* chromosome dimer resolution site dif and the type II **hybrid** site use the same **recombinase** but are independent of ArgR and PepA and show no site selectivity. This has led to the proposal that ArgR and PepA are responsible for the imposition of constraint. We describe here the characterization of a novel class of 'conditionally constrained' multimer resolution sites whose properties support this hypothesis. In the presence of ArgR and PepA, plasmids containing conditionally constrained sites are monomeric, but in their absence, extensive multimerisation is seen. A mutant ArgR derivative (ArgR110), which is defective in cer-mediated dimer resolution, remains able to prevent **plasmid** multimerisation by a conditionally constrained site. This implies that the accessory factors block **recombination** in trans rather than ensuring rapid multimer resolution. When the distance between the ArgR and XerCD binding sites in a conditionally constrained site was altered by a non-integral number of helical turns, the site became unconstrained. Constraint was restored by the insertion of a full helical turn.

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on STN

ACCESSION NUMBER: 95175546 EMBASE  
DOCUMENT NUMBER: 1995175546  
TITLE: Involvement of ArgR and PepA in the pairing of ColE1 dimer resolution sites.  
AUTHOR: Guhathakurta A.; Summers D.  
CORPORATE SOURCE: Department of Genetics, Downing Street, Cambridge CB2 3EH, United Kingdom  
SOURCE: Microbiology, (1995) 141/5 (1163-1171).  
ISSN: 1350-0872 CODEN: MROBEO  
COUNTRY: United Kingdom  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB Dimer formation and associated copy number depression is an important cause of multicopy **plasmid** instability. Natural multicopy plasmids employ site-specific **recombination** to convert dimers to monomers, thus maximizing the number of independently segregating molecules at cell division. Resolution of dimers of *Escherichia coli* **plasmid** ColE1 requires the **plasmid** cer site and at least four chromosome-encoded proteins: the XerC and XerD recombinases, and accessory factors ArgR and PepA. It has been suggested that ArgR has a role in the initial pairing of **recombination** sites and we describe here an attempt to detect this process *in vivo*. Our approach exploits a previous observation that a cer-like site known as the type II **hybrid** supports inter-molecular **recombination** and causes extensive multimerization of plasmids. We report that type-II-mediated multimerization can be repressed by a cer site in *cis* or in *trans* and propose that this is due to a physical interaction between the sites. If

this hypothesis is correct, suppression of multimer formation provides an assay of site pairing. Our results demonstrate that the putative pairing interaction is independent of the topological relationship of the sites and that both PepA and ArgR are involved. Although most **recombination**-deficient mutant derivatives of ArgR are unable to pair **recombination** sites, we have found two (ArgR110 and ArgR115(\*)) which retain pairing activity. The validity of the pairing hypothesis is discussed in the light of alternative explanations for our data.

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on STN

ACCESSION NUMBER: 95060450 EMBASE  
DOCUMENT NUMBER: 1995060450  
TITLE: The actinophage RP3 DNA integrates site-specifically into the putative tRNA(Arg) (AGG) gene of *Streptomyces rimosus*.  
AUTHOR: Gabriel K.; Schmid H.; Schmidt U.; Rausch H.  
CORPORATE SOURCE: Inst Genetik Mikrobiol Univ Munchen, Maria-Ward-Strasse 1A, 80638 Munchen, Germany  
SOURCE: Nucleic Acids Research, (1995) 23/1 (58-63).  
ISSN: 0305-1048 CODEN: NARHAD  
COUNTRY: United Kingdom  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB The temperate actinophage RP3 integrates site-specifically into the chromosome of *Streptomyces rimosus* R6-554. The phage attachment site **attP** and the **hybrid** attachment sites of the integrated prophage-attL and **attR**-were cloned and sequenced. The 54nt core sequence, common to all RP3 related attachment sites, comprises the 3' terminal end of a putative tRNA(Arg) (AGG) gene. **AttB** bears the complete tRNA gene which is restored in affL after integration. A 7.5 kb HindIII fragment, bearing **attP**, was used to construct an integrative plasmid to simulate the integration process *in vivo* and to localize the phage genes necessary for site specific integration. The int and xis genes were sequenced and compared to other **recombination** genes.

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on STN

ACCESSION NUMBER: 94107339 EMBASE  
DOCUMENT NUMBER: 1994107339  
TITLE: A DNA construct useful for specific chromosome loss in *Saccharomyces cerevisiae*.  
AUTHOR: Kawasaki H.; Ouchi K.  
CORPORATE SOURCE: Tokyo Research Laboratories, Kyowa Hakko Kogyo Co. Ltd., 3-6-6 Asahimachi, Machidashi, Tokyo 194, Japan  
SOURCE: Journal of Fermentation and Bioengineering, (1994) 77/2 (125-130).  
ISSN: 0922-338X CODEN: JFBIEX

COUNTRY: Japan  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB A GAL1-R/RS-RS cassette DNA was constructed to induce the loss of a specific chromosome in *Saccharomyces cerevisiae*. The cassette is composed of two specific **recombination** sites (RS) derived from the pSR1 plasmid of *Zygosaccharomyces rouxii*, and the site-specific **recombinase** gene, R, placed downstream of the GAL1 promoter. To delete a certain chromosome, the cassette was inserted into a site on that chromosome in a diploid cell by homologous **recombination** between

a DNA fragment cloned on the cassette and the relevant site on the chromosome. When the transformant was cultivated in galactose medium, elimination of the target chromosome occurred by R-promoted site- specific **recombination** between two unequal RS sites. Using this method, we demonstrated the loss of chromosomes III, V, VII and XV in heterozygous diploids. Aneuploids appeared at a frequency of 45-85% in the colonies examined after the induction of chromosome loss and were easily distinguished since they gave rise to smaller colonies than did the diploids. We confirmed that the aneuploids often duplicated the residual monosomic chromosome to restore the chromosome balance during mitotic growth. Such diploids formed colonies that were as large as the parent. This method is useful for conversion of heterozygous chromosomes into homozygous (or uniparental) ones in **hybrid** strains, and is also useful for chromosome loss mapping.

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ACCESSION NUMBER: 93310911 EMBASE  
DOCUMENT NUMBER: 1993310911  
TITLE: High-level heterologous gene expression in *Saccharomyces cerevisiae* from a stable 2 $\mu$ m plasmid system.  
AUTHOR: Ludwig D.L.; Ugolini S.; Bruschi C.V.  
CORPORATE SOURCE: Microbiology Department, Internat. Ct. Gen.  
Eng./Biotechnol., Padriciano 99, I-34012 Trieste, Italy  
SOURCE: Gene, (1993) 132/1 (33-40).  
ISSN: 0378-1119 CODEN: GENED6  
COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB The best candidate for a high-copy-number and mitotic stability expression system in yeast is the endogenous 2 $\mu$ m **plasmid**. Nevertheless, derivatives of the 2 $\mu$ m **plasmid** typically exhibit lower copy numbers and require selection for adequate maintenance within cells. We report the construction and utilization of an efficient heterologous gene expression system containing a 4.5-kb inducible expression cassette inserted into the 2 $\mu$ m **plasmid** and selected in cells utilizing a carrier **plasmid** which is subsequently lost via **FRT** /**Flp** **recombination**. The non-selectable 2 $\mu$ m **plasmid**, containing the cassette, was found to be stably maintained in cells, without selection, at high copy number. The dynamics of resolution and partitioning of this **plasmid** were analyzed during the course of 50 generations of growth under non-selective conditions. The heterologous lacZ reporter gene coding for  $\beta$ -galactosidase ( $\beta$ Gal) is driven by the **hybrid**, galactose-inducible promoter GAL10::pMF $\alpha$ 1. Upon induction,  $\beta$ Gal was secreted into the periplasm and culture supernatant at levels which could be detected directly from Coomassie blue-stained SDS-PAGE. Furthermore, **plasmid**-containing cells could be maintained directly on rich YPD medium and identified either by utilizing XGal or by observing inhibition of colony growth on YPGal solid medium. The cassette was designed for direct, high-level, inducible expression of cloned genes downstream from the MF $\alpha$ 1 signal sequence, with or without a C-terminal lacZ fusion. This **vector** represents the first demonstration of a non-selectable, mitotically stable, episomal **plasmid** system capable of expressing recombinant proteins at high levels. By supplanting the need for synthetic medium, this system could provide both an efficient and cost-effective means of generating recombinant protein at either the laboratory or large-scale level.

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ACCESSION NUMBER: 93301579 EMBASE

DOCUMENT NUMBER: 1993301579  
TITLE: Manipulation of transgenes by site-specific  
recombination: Use of Cre recombinase.  
AUTHOR: Sauer B.  
SOURCE: Methods in Enzymology, (1993) 225/- (890-900).  
ISSN: 0076-6879 CODEN: MENZAU  
COUNTRY: United States  
DOCUMENT TYPE: Journal; General Review  
FILE SEGMENT: 021 Developmental Biology and Teratology  
022 Human Genetics  
LANGUAGE: English

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ACCESSION NUMBER: 92132496 EMBASE  
DOCUMENT NUMBER: 1992132496  
TITLE: A phage T4 in vitro packaging system for cloning long DNA  
molecules.  
AUTHOR: Rao V.B.; Thaker V.; Black L.W.  
CORPORATE SOURCE: Department of Biology, 103 McCort Ward Hall, Catholic  
University of America, 620 Michigan Avenue,  
N.E., Washington, DC 20064, United States  
SOURCE: Gene, (1992) 113/1 (25-33).  
ISSN: 0378-1119 CODEN: GENED6  
COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
029 Clinical Biochemistry  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB Recombinant plasmid DNAs containing long DNA inserts that can be  
propagated in Escherichia coli would be useful in the analysis of complex  
genomes. We tested a bacteriophage T4 in vitro DNA packaging system that  
has the capacity to package about 170 kb of DNA into its capsid for  
cloning long DNA fragments. We first asked whether the T4 in vitro system  
can package foreign DNA such as concatemerized  $\lambda$ imm434 DNA and  
phage P1-pBR322 hybrid DNA. The data suggest that the T4 system  
can package foreign DNA as efficiently as the mature phage T4 DNA. We then  
tested the system for its ability to clone foreign DNA fragments using the  
P1-pBR322 hybrid vectors constructed by Sternberg [Proc. Natl.  
Acad. Sci. USA 87 (1990) 103-107]. E. coli genomic DNA fragments were  
ligated with the P1 vectors containing two directly oriented loxP  
sites, and the ligated DNA was packaged by the T4 in vitro system. The  
packaged DNA was then transduced into E. coli expressing the phage P1  
cyclization recombination protein recombinase to  
circularize the DNA by recombination between the loxP  
sites situated at the ends of the transduced DNA molecule. Clones with  
long DNA inserts were obtained by using this approach, and these were  
maintained as single-copy plasmids under the control of the P1  
plasmid replicon. Clones with up to about 122-kb size inserts were  
recovered using this approach.

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ACCESSION NUMBER: 87058717 EMBASE  
DOCUMENT NUMBER: 1987058717  
TITLE: In vitro packaging into phage T4 particles and specific  
recircularization of phage lambda DNAs.  
AUTHOR: Black L.W.  
CORPORATE SOURCE: Department of Biochemistry, University of Maryland Medical  
School, Baltimore, MD 21201, United States  
SOURCE: Gene, (1986) 46/1 (97-101).  
CODEN: GENED6

COUNTRY: Netherlands  
 DOCUMENT TYPE: Journal  
 FILE SEGMENT: 004 Microbiology  
 022 Human Genetics  
 047 Virology  
 LANGUAGE: English  
 AB Concatemeric phage  $\lambda$ imm434 DNA packaged in vitro into phage T4 particles produced plaques on a selective host. Moreover,  $\lambda$  DNA containing a pBR322 derivative flanked by the  $\lambda$  attL and attR sites could be specifically recircularized by excisive  $\lambda$  recombination to yield the pBR322 derivative. A host deficient in generalized recombination and containing a defective  $\lambda$ cIts prophage which provided Int and Xis proteins was the recipient for this plasmid derivative carried by T4. Such a T4- $\lambda$  hybrid may potentially allow almost one T4 headful of donor DNA (166 kb) to be packaged and recircularized.

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ACCESSION NUMBER: 81217092 EMBASE  
 DOCUMENT NUMBER: 1981217092  
 TITLE: Denaturation map of bacteriophage P1 DNA.  
 AUTHOR: Meyer J.; Stalhammar-Carlemalm M.; Iida S.  
 CORPORATE SOURCE: Dept. Microbiol., Biozent., Univ., CH 4056 Basel, Switzerland  
 SOURCE: Virology, (1981) 110/1 (167-175).  
 CODEN: VIRLAX  
 COUNTRY: United States  
 DOCUMENT TYPE: Journal  
 FILE SEGMENT: 047 Virology  
 022 Human Genetics  
 004 Microbiology  
 LANGUAGE: English  
 AB Electron microscopy of bacteriophage P1 plasmid DNA partially denatured by high pH in the presence of formaldehyde and mounted by the protein monolayer technique revealed a unique denaturation pattern. Alignment with the restriction cleavage map was obtained by comparing the melting pattern of plasmid DNA linearized by a single cut introduced by the restriction enzyme PstI with those of the large HindIII, BglII, and BamHI restriction fragments. Major A + T-rich segments center around map units 3, 21, 46, and 52. Other denatured regions are located around map units 29, 32 (within the invertible segment), 62, 88, and 96. The invertible segment of P1 DNA was precisely mapped within the Bam HI-5 and the BglII-5 DNA restriction fragments. The DNA segment melting first and therefore having the highest A + T content spans the P1-specific restriction modification region between map units 0 and 6 of the P1 genome. The P1-specific recombination site, loxP, is also located in a region of low DNA helix stability. The denaturation patterns of the DNA of a hybrid P1-R plasmid revealed IS1-mediated transposition of the r-determinant into a site adjacent to the most A + T-rich region on P1.

L16 ANSWER 22 OF 22 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
 ACCESSION NUMBER: 1982:208124 BIOSIS  
 DOCUMENT NUMBER: PREV198273068108; BA73:68108  
 TITLE: BACTERIO PHAGE P-1 SITE SPECIFIC RECOMBINATION 2.  
 RECOMBINATION BETWEEN LOX-P AND THE  
 BACTERIAL CHROMOSOME.  
 AUTHOR(S): STERNBERG N [Reprint author]; HAMILTON D; HOESS R  
 CORPORATE SOURCE: CANCER BIOL PROGRAM, NATIONAL CANCER INST, FREDERICK CANCER  
 RES CENT, FREDRICK, MD 21701, USA  
 SOURCE: Journal of Molecular Biology, (1981) Vol. 150, No. 4, pp. 487-508.

DOCUMENT TYPE: Article  
FILE SEGMENT: BA  
LANGUAGE: ENGLISH

AB The events associated with **recombination** between **loxP**, a site in phage P1 EcoRI fragment 7, and **loxB**, a site in the bacterial [Escherichia coli] chromosome, are described. When fragment 7 is in a phage  $\lambda$  **vector**, the product of **loxP** + **loxB** **recombination** is the integration of the  $\lambda$ -P1:7(**loxP**) DNA as prophage in the bacterial chromosome and the production of 2 new **hybrid** sites, **loxR** and **loxL**, flanking the prophage. Lambda phages containing **loxL**, **loxR** and **loxB** sites were isolated and used to measure **recombination** frequencies between all 4 **lox** sites in paired phage crosses. **loxP** and **loxL** sites were much better substrates for **recombination** than are **loxR** and **loxB** sites. Both partners in a **recombination** reaction must contain either **loxP** or **loxL** sites for **recombination** to be efficient. These results can explain all of these findings relating to the **recombination** between  $\lambda$ -P1:7 DNA and the bacterial chromosome. In particular, integration of that DNA into **loxB** is low because **loxP** + **loxB** **recombination** is inefficient, and excision of the resulting prophage DNA is low because **loxL** + **loxR** **recombination** is inefficient. In contrast, integration of  $\lambda$ -P1:7 DNA into a chromosome already containing a  $\lambda$ -P1:7 prophage at **loxB** is relatively high and is always accompanied by the excision and subsequent loss of the original prophage DNA. This process was called integration by prophage displacement, and it can be accounted for by postulating that 2 **lox recombination** events are involved (**loxP** + **loxR** and **loxL**), both of which are more efficient than is **loxP** + **loxB** **recombination**. Analysis of the physical structure of the various  $\lambda$  **lox** phages indicates that **loxP**-containing DNA can integrate into **loxB** in both possible orientations, and that the **loxP** + **loxB** crossover point is located within a 100 base-pair region of P1 BamHI fragment 9, a subfragment of EcoRI fragment 7. The **loxB** site was mapped to a region of the E. coli chromosome between **tolC** (66 min) and **dnaG** (67 min).